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Comparison between aminopeptidase and trypsin activity in blood-fed females of *Aedes aegypti*

by

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With 3 figures

Abstract

Female mosquitoes show a low level of aminopeptidase activity throughout adult life. After a blood meal however it rises by a factor of six within 24 hours, reaching the maximum together with tryptic activity. After the digestion period the residual level in the midgut is restored. Over 50% of the total enzyme activity is localized in the ectoperitrophic space, i.e. between midgut epithelium and the peritrophic membrane, whereas about 40% is detected within the epithelial cells. The rest of only 10% is found within the blood bolus. Consequently, between the midgut epithelium and the peritrophic membrane a compartment is established for optimal digestion.

INTRODUCTION

Haematophagous insects require blood meals as a source of protein exclusively for reproduction as in mosquitoes, or for growth and reproduction as in *Rhodnius* (Wigglesworth 1965). The protein is digested by the midgut in a short time (Briegel & Lea 1975) and the resulting amino acids and peptides are used for vitellogenin synthesis in the fat body. Thus digestion represents one of the initial steps in the processes of reproduction.

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The synthesis of proteolytic enzymes in the midgut has been studied by GOODING (1973) and by BRIEGEL & LEA (1975). Trypsin acting as an endopeptidase was found to be the major component of the proteolytic activity. Little is known however about the activity of the exopeptidases in general and about their relation to tryptic activity in particular. This led us to investigate the physiological levels of aminopeptidase activities throughout the lifecycle of a mosquito and in response to blood meals.

MATERIALS AND METHODS

Aedes aegypti (strains Segemaganga and Ifakara) were kept under standards conditions as described by BRIEGEL & LEA (1975) and after adult eclosion they were provided with sucrose *ad lib*. For blood meals, measured amounts of heparinized rat blood (20 units of heparin per 1 ml of blood) were injected into anaesthetized females through the anus, i.e. by enema.

Midguts were dissected and freed of Malpighian tubules, homogenized in 0.05 M phosphate buffer (pH 7.2) with a sonicator microtip (Branson) and subsequently frozen until analysis (− 20° C).

The assay for aminopeptidase activity of BERGMEYER (1962) was slightly modified: the substrate volume was reduced to 1 ml and the absorbance of the naphtylamide-Echtrot-G-salt complex was read at a wavelength of 500 nm. Trypsin activity was measured according to HUMMEL (1959), using TAME as a substrate. The protein content was determined by the method of BRAMHALL *et al* (1969). Inhibition experiments were carried out by preincubation of the homogenate with TLCK (SHAW *et al.* 1965) or buffer at room temperature for at least an hour before enzyme activity was tested again with TAME and leucyl-naphtylamid.

RESULTS

In order to assess optimal conditions for aminopeptidase activity, the pH of the assay solution was varied. The activity was found to have maxima at ph 7.2 and ph 8 respectively. A preference of aminopeptidase for specific sites within a protein was tested with naphtylamide coupled to 15 different aminoacids. Comparison of the results revealed the highest affinity of aminopeptidase for leucin (100%), medium affinity to alanin (76%), arginin (67%), methionin (55%), lysine (49%) and a very low affinity (< 10%) to the remaining amino acids (tyrosine, serine, glutamic acid and proline).

The high affinity of aminopeptidase to arginine suggested a possible interference by trypsin which specifically recognizes arginine and lysine residues for hydrolytic action. To test this possibility, assays for aminopeptidase and trypsin were performed with either trypsin (bovine), leucineaminopeptidase (hog) or midgut homogenate (mosquitoes). In addition, the specific trypsin inhibitor TLCK was added to some of the samples to ensure total inhibition. Table 1 clearly demonstrates that trypsin had no effect on either arginine or leucine residues present in the substrates.

We have analyzed larval and pupal homogenates of *Aedes aegypti* in daily intervals as well as imaginal midguts from eclosion up to an age of 50 days (collections at 5-day intervals). A dramatic increase of activity during the first 3 days of larval life is followed by a drop before and during metamorphosis, reaching the adult residual value. In females not receiving blood meals this residual level is maintained throughout life time showing a slow decline later in life (Fig. 1). The response of the midgut to a blood meal was tested during the first days after eclosion following injection of 4 µl of blood plasma. At

TABLE 1.

Effect of trypsin on the aminopeptidase activity of Aedes aegypti *with respect to the substrates arginine- and leucinenaphtylamide. TLCK, a trypsin inhibitor was added to distinguish between trypsin and aminopeptidase activity. The control for trypsin activity with its substrate (TAME) was also treated with TLCK.*

	Aminopeptidase-activity		Trypsin-activity	
	without TLCK	with TLCK	without TLCK	with TLCK
Trypsin	− − −	− − −	+ + +	− − −
LAP	− − −	− − −	− − −	− − −
Homogenate. . . .	+ + +	+ + +	+ + +	− − −

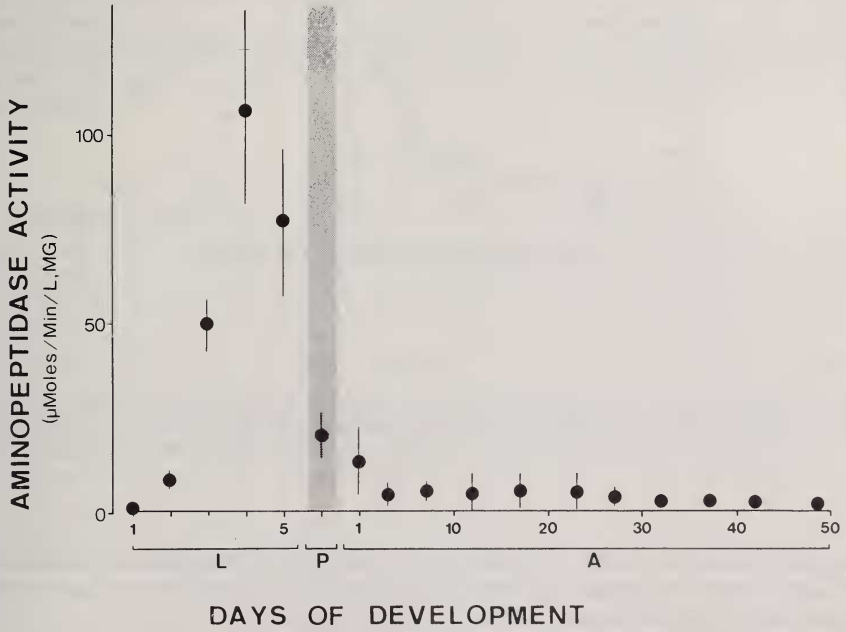

FIG. 1.

Changes of aminopeptidase activity through larval (L), pupal (P) and adult (A) lifespan, measured at the times indicated. Larval and pupal enzyme determinations (n = 5 each) were done on whole body homogenates whereas in the adults the midgut tissue only was assayed (n = 10 each). S.E. are given by bars unless they are too small to be shown.

intervals of 4 hours 5-10 animals were dissected and analyzed for aminopeptidase activity, trypsin activity and protein content. Fig. 2 demonstrates a sixfold increase of aminopeptidase activity within the first 24 hours after the blood meal. With a delay of 3-6 hours tryptic activity also increases. The times of highest activity for these two

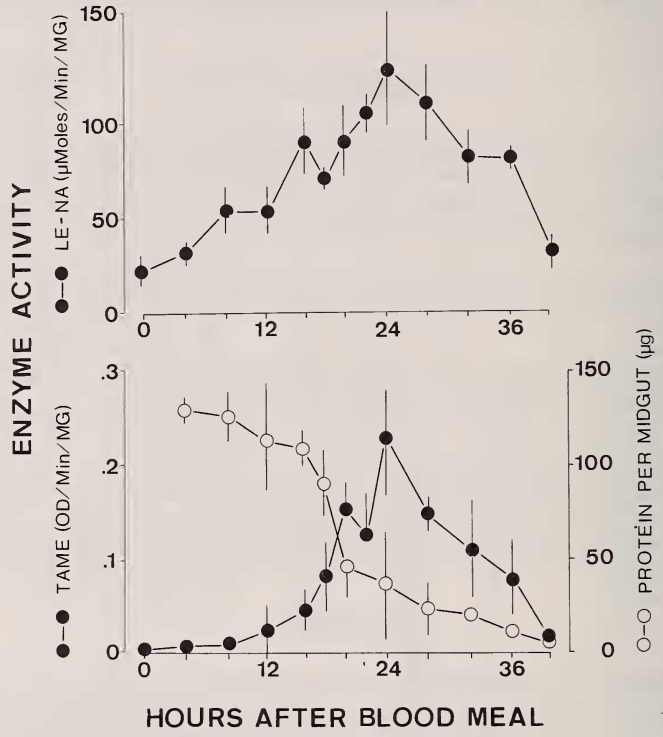

FIG. 2.

Effect of blood plasma (4 μl) injected into *Aedes aegypti*. 4-6 females were dissected and assayed for aminopeptidase activity with the substrate leucinenaphtylamid (LE-NA) and for trypsin activity with TAME. Protein content was determined per midgut. Bars represent S. E.

enzymes coincide at 24 hours. About 10-12 hours later when digestion is completed, aminopeptidase is excreted in an active form while the residual activity in the midgut is maintained throughout the following hunger period.

To test the mode of enzyme production various amounts of protein were injected into *Aedes aegypti*; 16-28 hours after injection aminopeptidase and trypsin activity were measured. The results in Tab. 2 indicate a positive correlation between protein content and maximal enzyme activity.

Up to this point enzyme activities were measured in total midgut homogenates only. To pinpoint the localization of the enzyme activity further, the midgut epithelium,

TABLE 2.

Positive correlation between size of the blood meal and aminopeptidase activity in Aedes aegypti. *Average enzyme activity (Means ± S.E. for 5-9 determinations) between 16 and 28 hrs after blood meal is given.*

	Hours after blood meal			
	16	20	24	28
Plasma 1 µl	44.7 ± 12	43.5 ± 6	44.2 ± 13	
Blood 1 µl	61.5 ± 6	61.2 ± 13	50.5 ± 6	
Blood 3 µl		110.7 ± 15	107.9 ± 13	86.1 ± 25
Blood 5 µl		119.8 ± 15	126.2 ± 11	130.4 ± 12

ectoperitrophic space and blood bolus were assayed separately (Fig. 3). Aminopeptidase activity was present in small amounts in the blood bolus (10%) whereas over 50% was located in the narrow space between the peritrophic membrane and the midgut epithelium, the so-called ectoperitrophic space. The epithelial cells of the midgut contained about 40% of enzyme activity. A similar situation has been observed previously for tryptic activity.

DISCUSSION

A dramatic increase of aminopeptidase activity is observed during 24 hours after ingestion of a blood meal. The activity reaches its maximum in accordance with tryptic activity (BRIEGEL & LEA 1975). For both enzymes the maximal level is positively correlated with the amount of protein ingested (BRIEGEL & LEA 1975). The coincidence of the time of the highest activity for both enzymes might indicate a concurrent need for both enzymes in the digestive process. Unexpectedly, there is a temporal delay of 3-6 hours between the early increase of aminopeptidase activity and the rise of tryptic activity (Fig. 2). This is contrary to the functional assumption of endopeptidases (such as trypsin) producing small peptides which serve as substrates to exopeptidases. Possibly there are enough peptides present in the blood to allow immediate action of exopeptidases. If so this might explain the fast release of EDNH after the blood meal (LEA 1967), which according to CHANG & JUDSON (1977) was triggered by initial products of digestion.

The highest enzymatic activities of aminopeptidase (Fig. 3) as well as trypsin (unpubl. observ.), are localized in the slightly yellow fluid contained in the narrow ectoperitrophic space (Fig. 3). This fluid provides a favourable medium for proteolytic action because of its proximity to both, the protein reserves (blood bolus) and the absorbing epithelium. This suggests a function of the peritrophic membrane, establishing a compartment with concentrated enzymatic activity which enables digestion to proceed from the periphery to the core of the blood bolus.

Large quantities of aminopeptidase are synthetized throughout the larval life span. During metamorphosis the enzyme activity declines sharply, confirming the results of

Spiro (1974) for *Culex pipiens*. A considerable, fairly constant residual activity remains after adult eclosion. It slowly declines only in the aging female mosquito.

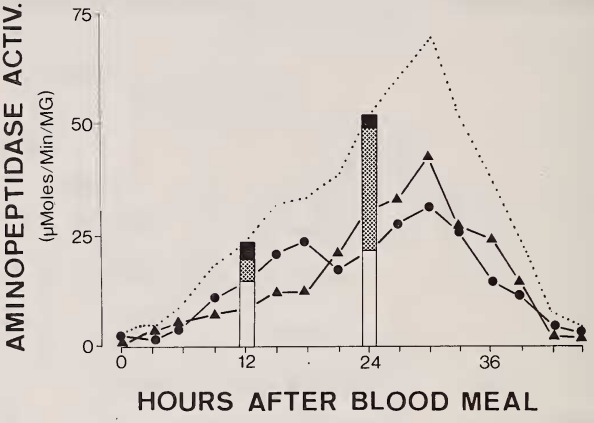

Fig. 3.

Effect of a blood meal on aminopeptidase activity in different parts of the midgut. 5 *Aedes aegypti* female midguts were separated into midgut epithelium (●———●) and content (▲———▲). In addition, 5 midguts were dissected at 12 and 24 hrs after blood ingestion: midgut epithelium (▭), digestive juice (▨) and blood bolus (■).

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